Preparation of Modified Selective Medium for Isolation of *Enterococcus faecalis* in Pure Culture from Heavy Sources (Rapid Diagnosis)

Jawad Kadhim Tarrad AL-Khafaji
Department of Microbiology, College of Medicine, University of Babylon, Hilla, Iraq

Abstract

Subject: This research focuses on the preparation of a new modified selective medium for the primary isolation of *Enterococcus faecalis* in pure culture from heavy and clinical sources. **Materials and Methods:** The modified selective medium was prepared and named as Enterococcus Jawad (EJ)–Medium. The new medium was prepared by adding 0.4 g of sodium azide, 2 mL of 0.1% crystal violet (CV), and 60 g of sodium chloride in a liter of Brain-Heart Infusion Agar (BHIA), and the pH of the medium was adjusted to 8.6. The primary isolation of *E. faecalis* from heavy bacterial sources and clinical infections was performed by culturing all collected samples on EJ-medium and comparing them with other approved selective media, such as Azide-BHIA, CV-blood agar, and Enterococcus selective agar (ESA), to estimate the ability of these media in isolating *E. faecalis* (during primary isolation) in pure culture. **Results:** The results showed that the EJ-medium had a high rate of isolation, 100%, of *E. faecalis* in pure culture from heavy sources (human feces and sewage water) and from clinical sources (urine, bedsore, and blood) when compared with other culture media that were already used for isolation of this organism. The Azide-BHIA and CV-blood agar showed no high purity in an initial isolation of the organism. **Conclusions:** From these results, we concluded that the EJ-medium is highly efficient in the primary isolation of *E. faecalis* in pure culture by one step from different environmental and clinical sources; thus, it does not need to be diagnosed by classical methods that are expensive and consume a long time. Since Azide-BHIA, CV-blood agar, and ESA were not exactly selective media for the isolation of *E. faecalis* in pure cultures from heavy sources, their use was limited in clinical cases.

Keywords: *Enterococcus faecalis*, modified selective media, purified isolation

Introduction

The enterococci are primarily commensal residents in the intestine of humans and animals. The species *Enterococcus faecalis* is a common inhabitant of the human intestinal microflora and the genitourinary tract of men and women.[1] The species is recognized as an important causative agent of health care–associated infections, including urinary tract infections (UTIs), postsurgical wound infections, bacteremia, endocarditis, meningitis, and neonatal sepsis.[2]

E. faecalis is implicated in about 10% of cases and up to 16% of nosocomial UTI. The species is responsible for a significant proportion of cases of bacterial endocarditis, accounting for 5–20% of reported series. Bacteremias due to this organism are more common than infective endocarditis. Bedsores and wound infections often occur in elderly patients with serious underlying medical conditions or in immunocompromised individuals who have undergone antimicrobial therapy.[3]

Because of their ability to resist most antibiotics and antiseptics, the strains of *E. feacalis* are found on patients' beds, medical devices, hospital floors, and water polluted with these bacteria, creating a health problem. In the past decade, *E. faecalis* has become one of the main pathogens of hospital-acquired infections, especially among patients who have

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Address for correspondence: Dr. Jawad Kadhim Tarrad AL-Khafaji, Department of Microbiology, College of Medicine, University of Babylon, Babylon Province, Hilla, Iraq.
E-mail: dr.jawad66@yahoo.com

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been a long time in the hospital. These bacteria cause many human diseases, such as UTIs, wound infections, bedsores, endocarditis, and other infections.[4] Therefore, they require rapid and accurate diagnostic methods, the most important of which is the primary isolation of *E. faecalis* from different sources.

The aim of the study was to prepare a new modified selective medium for pure isolation and rapid identification of *E. faecalis* in a short time.

Materials and Methods

Preparation procedure of the new medium

Crystal violet (0.1 g; CV; Oxoid/United Kingdom) was dissolved in 100 mL of distilled water; then, it was sterilized by filtration using a 0.22 nm membrane filter. The solution was stored in a dark bottle at 4°C.

Sodium azide (0.4; Oxoid/United Kingdom) and sodium chloride (60 g) were added to 1000 mL of Brain-Heart Infusion Agar (BHIA; Oxoid/United Kingdom); then, they were sterilized by autoclave for 15 min.

After autoclaving, 5 mL of fresh human blood and 2 mL of 0.1% CV solution were added to the melted medium at 45°C with gentile mixing.[3,5-7]

The pH of the medium was adjusted to 8.6 with sodium hydroxide (0.1 M, Oxoid/United Kingdom) solution.[8,9]

The new medium was named EJ-medium.

Collection of specimens

The study was conducted at Teaching General Hilla Hospital, Babylon governorate. Two hundred fifty samples were collected from different sources. Of these, 125 stool samples were collected from the patients who were admitted to the hospital. The samples were taken from the patients by standard procedures as described by Collee *et al*.,[5] and 125 samples were collected from sewage water of the hospital and AL-Uhodyia canal at Hilla city by standard methods described by Tang and Stratton.[10]

Different specimens were also collected from the same patients: 30 samples of urine from UTI cases, 25 samples of patients with bedsores, and 10 blood samples from patients with endocarditis. These samples were collected by the procedures mentioned by Tille[4] and Collee *et al*.[5]

Comparative study

The primary isolation of *E. faecalis* from heavy sources was performed by culturing each collected sample on EJ-medium separately, and other culture media such as CV-blood agar, Azide-BHIA, and ESA medium were inoculated with the samples.

The estimation of the ability of EJ-medium in the isolation of *E. faecalis* in pure culture was conducted in the following manner: (i) The bacterial growth appeared on the EJ-medium compared with on other selective culture media. (ii) The mixed cultures that developed on other culture media were again inoculated on EJ-medium.

Standard biochemical tests mentioned in approved microbiological references[4,7,11] were performed to confirm the bacterial isolates as *E. faecalis*.

Results and Discussion

Previously, the primary isolation of *E. faecalis* was carried out by using some selective media, such as ESA medium, or using blood agar medium and BHIA that are supplemented with some bacterial inhibitors either with CV only[6] or with sodium azide only.[7]

These media are not exactly selective media for the isolation of *E. faecalis* in pure cultures. This fact explains that the media contain either only Na-Azide that leads to the inhibition of Gram-negative bacteria only or only CV that results in the inhibition of Gram-positive bacteria only; therefore, some species of other bacteria can be grown on these media through primary isolation.

The new medium, EJ-medium, is composed of enrichment base medium (BHIA-base) that is supplemented with both the inhibitors, Na-azide and CV, so that both Gram-negative and Gram-positive bacteria are inhibited, except *E. faecalis*, which is already resistant to CV. The new medium also contains a high concentration of salt, and the alkaline condition is not a favorable environment to grow most types of microorganisms, so most contaminants can be removed in the first step.

The new modification, including BHIA-base medium, was chosen rather than other media because it enriched the medium. Both the inhibitors, the sodium azide and the CV, were added to the BHIA-base in the same medium, instead of adding each inhibitor separately in ordinary medium. Fresh human blood that had not been previously added was added to the new medium. Another new modification involved increasing the salt concentration of the medium by making the final concentration of sodium chloride 6.5% and creating an alkaline environment with pH 8.6.

Our results show that the EJ-medium is highly significant in clearance form in 100% pure isolation of *E. faecalis* from heavy sources (stool and sewage water) and from clinical sources (urine, bedsore, and blood) when compared with classical culture media that are already used for the isolation of this organism. The Azide-BHIA and CV-blood agar show mixed cultures that indicate the growth of bacterial contaminants and low purity in the isolation of *E. faecalis* from different sources; see Table 1 given next.

EJ-medium showed high efficiency in the isolation of *E. faecalis* in pure culture from sources of high contamination and clinical infections. This means that the new medium contains an enriched medium that promotes the growth of *E. faecalis*, and it contains both inhibitors,

Table 1: Purity of bacterial growth on EJ-medium compared with other culture media for isolation of *E. faecalis* from different sources

Samples		Growth of *E. faecalis* on			
Type of sample	No. of samples	EJ-medium	Azide-BHI	CV-blood agar	ESA
Stool	125	125 pure	125 mixed	125 mixed	90 pure
					35 mixed
Sewage water	125	125 pure	125 mixed	125 mixed	75 pure
					50 mixed
Urine	30	10 pure	2 pure	1 pure	10 pure
		20 NG	28 mixed	29 mixed	20 NG
Bedsore	25	25 pure	9 pure	6 pure	25 pure
			14 mixed	10 mixed	
			2 NG	9 NG	
Blood	10	2 pure	5 mixed	7 mixed	2 pure
		8 NG	5 NG	3 NG	8 NG

BHI: Brain-Heart InfusionCV: crystal violet, EJ: Enterococcus Jawad, ESA: Enterococcus selective agar, NG: no growth

so the Gram-positive and Gram-negative bacteria cannot grow on this medium. In addition, the pH of the medium and high sodium chloride concentration does not permit the growth of contaminants, such as bacteria and fungi, except *E. faecalis*, which is resistant to both inhibitors and can grow under these harsh saline and alkaline conditions. Therefore, the EJ-medium is considered as an excellent selective medium for the isolation of this bacterium from different sources either environmentally or clinically.

The new medium can be easily applied in different fields.

1. This new medium can be used by researchers and postgraduate students who are involved in the easy isolation and diagnosis of these bacteria.
2. It can be used by laboratory workers in our country's hospitals to isolate and diagnose diseases caused by these bacteria.
3. The new medium can be used in health and epidemiological studies in the community without much hassle.

In brief, the EJ-medium has many advantages, such as its materials are easily available; it is cheap, easy to use, easy to prepare, and highly efficient in the isolation of *E. faecalis* without any microbial contamination. Because this medium is highly efficient and gives 100% pure isolates, it does not need to be diagnosed by conventional methods (does not require chemical and physiological tests), which are costly and exhausting for academic researchers. It also reduces the material cost of the state, especially in the health and research sectors. Time is shortened, so researchers who use this medium do not need more time (is not time-consuming) for the diagnosis of this organism, but they diagnose it in the first step (in primary isolation); the bacteria can be obtained in a pure step and do not need other diagnostic tests to be performed. The new medium also reduces the effort exerted by the concerned parties in the preparation of the chemical solutions, the reagents, the culture media, and other materials to diagnose this bacterium.

Conclusions

We can conclude the following points: (i) EJ-medium is excellent for the isolation of *E. faecalis* in pure culture from environmental sources and clinical cases. (ii) Azide-BHIA, CV-blood agar, and ESA are not suitable media in primary isolation of the organism from heavy sources but can be used in clinical cases with limited things.

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Conflicts of interest

There are no conflicts of interest.

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